

### **DETAILED ACTION**

1. The amendment filed on 03/16/2010 has been entered and fully considered. Claims 1-18 are pending, of which Claims 1, 6, 7, and 18 are amended.

### ***Response to Amendment***

2. In response to amendment, the examiner modifies rejection over the prior art established in the previous Office action.

### ***Claim Rejections - 35 USC § 103***

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
4. **Claims 1, 2, 4-6 and 18** are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita et al. (Electrophoresis, 1998) (Tsugita) in view of Covey et al. (US Patent 5,952,653) (Convey) and Xu et al. (Analytical Biochemistry, 1997) (Xu).

In regard to Claims 1 and 18, Tsugita teaches a method of analyzing C-terminal sequence of a peptide by means of mass spectrometry.

Tsugita teaches pretreatment step using acetic anhydride react with the original dried peptide to acetylate the N-terminus and form an oxazolone at the C-terminal carboxyl group (see page 930, right col. 3<sup>rd</sup> paragraph). While Tsugita does not specifically teach acetylation of the side chain of lysine residue, acetylation of the side chain of lysine residue is the inherent result of reacting acetic anhydride with peptide that has lysine residue.

Tsugita teaches a step of allowing acetic anhydride to act on the original dry peptide in the presence of pentafluoropropionic methyl ester (PFPM<sub>e</sub>) to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring (see page 930, right col. 3<sup>rd</sup> paragraph). PFPM<sub>e</sub> is similar to pentafluoropropionic acid (PFPA) in structure and reaction. Tsugita also teaches using PFPA in the cleavage of predetermined position of the peptide (see page 932, left col. 4<sup>th</sup> paragraph). It would have been obvious to ordinary skill in the art to substitute PFPM<sub>e</sub> with PFPA in the step of releasing the C-terminal amino acids in Tsugita method, because these compounds

have similar structures and similar effects. Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). In that regard, although the formation of the 5-oxazolone ring and the cleavage of 5-oxazolone ring are carried out at the different temperatures, it would have been obvious to one of ordinary skill in the art to optimize the temperature of known reactions by routine experimentation so that both reactions can be carried out in parallel.

Tsugita teaches hydrolysis treatment step by allowing water molecules to act on the C-terminal-deleted peptides in the presence of catalytic amount of dimethylamino ethanol (DMAE), a tertiary amine compound (see page 930, right col. 3<sup>rd</sup> paragraph).

Tsugita teaches measuring the decreases in molecular weight associated with the successive release of the C-terminal amino acids by mass spectra (see Table 3).

Tsugita does not specifically teach allowing trypsin to act on the sample to cleave peptide for mass spectrometer analysis. Tsugita teaches that highly specific proteases have been used for specific fragmentation in the peptide-mass fingerprinting technique (see page 931, left col. 2<sup>nd</sup> paragraph). Trypsin is one of highly specific proteases commonly used. Covey teaches using trypsin to cleave peptide for mass spectrometry analysis (see abstract). Covey further teaches that the tryptic fragments ions are predominantly doubly charge cationic species, because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2, lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is single charged (see col. 5, lines 58-60). At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin to cleave the peptide before mass spectrometry analysis, as taught by Covey, in the method of Tsugita, because smaller peptides are easier to analyze by mass spectrometry.

Tsugita does not teach the protocol of analyzing mass spectra by comparing the peaks of cationic species with the peaks of the anionic species as described in the

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steps 1-9 in the instant claim. As has been discussed above, Covey teaches that tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2, lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is single charged (see col. 5, lines 58-60). The method of analyzing mass spectra according to the expected charges of the species is well known in the art.

Xu teaches that fragmentation patterns in positive mode and negative mode are complementary for the elucidation of the peptide chain sequence (see page 10-13, *MALDI-PSD Analysis*). Xu further teaches correlating peaks that only different in a chemical group, e.g. OH (mass of 18) or N-acetyl glucosamine (MurNAC mass of 203) because of the reaction (see page 10, right col.). Xu's teaching is similar to the criteria 5a-1, 5a-2, 5a-3, 5b-1, 5b-2, 5b-3 of the instant claim.

Applicant is advised that the rationale to support a conclusion that the claim would have been obvious is that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art. (see KSR, 550 U.S. at \_\_\_, 82 USPQ2d at 1395) (see MPEP 2143). In that regard, one of ordinary skill in the art could have utilized the charge difference between normal tryptic fragments and C-terminal tryptic fragment as taught by Covey, and correlated the fragmentation patterns in positive mode and negative mode in relative intensity and further correlated with the special group difference due to chemical reaction, as taught by Xu to elucidate the peptide chain sequence by calculating the mass difference between the C-terminal successive deleted peptides as taught by Tsugita, with the predictable result.

In regard to Claim 2, it is well known in the art that spiked noise in mass spectra usually has narrower full-width of half maximum than normal peak of signal. Therefore, removing peaks of spiked noise based on full-width of half maximum would have been obvious to ordinary skill in the art. The peak smoothing and smoothing algorithms are

also well known in the art. Therefore, peak smoothing would have been obvious to ordinary skill in the art.

In regard to Claim 4, the normal tryptic fragments carry two positive charges as taught by Covey. Under the same reason, when C-terminal fragment has arginine at the C-terminal (CFAC), it will also carry two positive charges. However, just like other C-terminal fragments, CFAC will also have an adjacent fragment peak that has molecular weight difference from CFAC equals formula weight of natural chain amino acid or acylated amino acid as taught by Tsugita. It would have been obvious to ordinary skill in the art to use the criteria of adjacent peaks to judge if the strong cationic peak is CFAC based on teaching of Tsugita and Convey.

In regard to Claim 5, Tsugita teaches measuring the decrease in molecular weight associated with successive release of the C-terminal amino acids (see Table 3). Tsugita teaches using MALDI-TOF-MS for the measurement (see page 931, left col. 2<sup>nd</sup> paragraph). Xu teaches considering fragmentation patterns in both cationic species (positive mode) and anionic species (negative modes) (see abstract). At the time of the invention, it would have been obvious for ordinary skill in the art to consider both cationic species and anionic species as taught by Xu in Tsugita's method to obtain the peptide sequence, because Xu specifically teaches that the fragmentation in positive mode and negative mode are complementary for elucidation of the peptide sequence.

In regard to Claim 6, Tsugita teaches a process for releasing the C-terminal amino acids successively. Tsugita teaches pretreatment step using acetic anhydride and acetic acid vapor react with the dried peptide at 60°C to acetylate the N-terminus and form an oxazolone at the C-terminal carboxyl group (see page 930, right col. 3<sup>rd</sup> paragraph). Tsugita does not specifically teach acetylation of the side chain of lysine residue. Acetylation of the side chain of lysine residue is the inherent result of reacting acetic anhydride with peptide that has lysine residue.

Tsugita teaches a step of allowing acetic anhydride vapor to act on the dry peptide in the presence of pentafluoropropionic methyl ester (PFPM<sub>e</sub>) vapor to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring at 5°C (see page 930, right col. 3<sup>rd</sup> paragraph). PFPM<sub>e</sub> is similar to pentafluoropropionic

acid (PFPA) in structure and reaction. Tsugita also teaches using PFPA in the cleavage of predetermined position of the peptide (see page 932, left col. 4<sup>th</sup> paragraph). It would have been obvious to ordinary skill in the art to substitute PFPMc with PFPA in the step of releasing the C-terminal amino acids. Tsugita allows the reaction at 5°C. The court has held that [W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation (*In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)). In that regard, although the formation of the 5-oxazolone ring and the cleavage of 5-oxazolone ring are carried out at the different temperatures, it would have been obvious to one of ordinary skill in the art to optimize the temperature of known reactions by routine experimentation so that both reactions can be carried out in parallel.

Tsugita does not literally teach removing the remaining alkanolic acid anhydride and perfluoroalkanoic acid in a dry state at the end of C-terminal cleaving reaction. However, this removing step at the end of the reaction is inherent part of the reaction step, because when the reaction is complete, the remaining reagents should be removed. Tsugita teaches hydrolysis treatment step by allowing water molecules to act on the C-terminal-deleted peptides in the presence of dimethylamino ethanol (DMAE), a tertiary amine compound. (see page 930, right col. 3<sup>rd</sup> paragraph). Again, the removing remaining basic nitrogen-containing organic is inherent part of the reaction step.

Tsugita does not specifically teach allowing trypsin to act on the sample to cleave peptide for mass spectrometer analysis. Tsugita teaches that highly specific proteases have been used for specific fragmentation in the peptide-mass fingerprinting technique (see page 931, left col. 2<sup>nd</sup> paragraph). Trypsin is one of highly specific proteases commonly used. Covey teaches using trypsin to cleave peptide for mass spectrometry analysis (see abstract). Covey further teaches that the tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not contain an arginine or a lysine is signally charge (see col. 5, lines 58-60). Since the side chain of lysine is protected by acetylation treatment as discussed above, only arginine site will be cut by

trypsin. At the time of the invention, it would have been obvious to one of ordinary skill in the art to use trypsin to cleave the peptide before mass spectrometry analysis as taught by Covey in the method of Tsugita, because smaller peptides are easier to analyze by mass spectrometry.

Removing trypsin at the end of the reaction is inherent part of the reaction. Desalting treatment is commonly used for changing buffer or removing buffer solution component. It would have been obvious to ordinary skill in the art to use desalting treatment to remove the trypsin from the buffer solution to stop the reaction. Drying sample is a required step before performing MALDI-TOF-MS.

Tsugita teaches using MALDI-TOF-MS for measuring the molecular weight of the peptide fragments (see page 931, left col. 2<sup>nd</sup> paragraph). Tsugita does not teach analyzing mass spectra by comparing the peaks of cationic species with the peaks of the anionic species. As has been discussed above, Covey teaches that tryptic fragments ions are predominantly doubly charge cationic species because arginine and lysine are both very basic and each picks up a positive charged proton in solution (see col. 2. lines 63-67; Col. 3, lines 39-42), and the C-terminal tryptic fragment not containing an arginine or a lysine is singly charge (see col. 5, lines 58-60). The method of analyzing mass spectra according to the expected charges of the species is well known in the art. Xu teaches that fragmentation patterns in positive mode and negative mode are complementary for the elucidation of the peptide chain sequence (see abstract). It would have been obvious to one of ordinary skill in the art to utilize the charge difference between normal tryptic fragments and C-terminal tryptic fragment as taught by Covey and correlate the fragmentation patterns in positive mode and negative mode as taught by Xu to elucidate the peptide chain sequence by means of MALDI-TOF-MS as taught by Tsugita.

5. **Claim 3** is rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu, as applied to claims 1-2 and 4-6 above, and further in view of Harris et al. (Rapid Communications in mass spectrometry, 2002) (Harris).

In regard to Claim 3, Tsugita in view of Covey and Xu do not teach using the fragments of trypsin autolysis as the internal standard to calibrate mass spectra. The

molecular weights and charges of peptide fragments derived from the trypsin autolysis are well known in the art. Harris teaches using trypsin autolysis fragments as mass calibrants in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (see abstract). Therefore, it would have been obvious to ordinary skill in the art to modify Tsugita-Convey-Xu's method by calibrating mass spectra using the peptide fragments of trypsin autolysis, as taught by Harris.

6. **Claims 8-17** as dependent on Claim 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tsugita in view of Covey and Xu.

In regard to Claims 8 and 9, Tsugita teaches that 20% acetic anhydride is used in the first step of the procedure for applying N-acetylation protection to the N-terminal of the protein and for forming oxazolone at C-terminal of the protein and 5% PFPMc is used in the second step to react with oxazolone (page 930, right col. 3<sup>rd</sup> paragraph). Tsugita does not specifically teach maintaining acetic anhydride in the second step. However, since the function of acetic anhydride is to form oxazolone at C-terminal for perfluoroalkanoic acid to act on in the second step, it would have been obvious to ordinary skill in the art to recognize that maintaining the concentration of acetic anhydride in the second step may benefit the reaction. Therefore, modified method of Tsugita teaches using symmetric anhydride of linear  $\alpha$ -chain alkanic acid having 2 carbons (acetic anhydride) for the formation of 5-oxazolone and subsequently release of C-terminal amino acids in association with cleavage of the 5-oxazolone ring (see page 930, right col. 3<sup>rd</sup> paragraph).

In regard to Claim 10, Tsugita teaches using acetic anhydride as the alkanic acid anhydride (see page 930, right col. 3<sup>rd</sup> paragraph).

In regard to Claim 11, the pK<sub>a</sub> of PFPA is in a range of 0.3 to 2.5.

In regard to Claim 12, PFPA has 3 carbon atoms.

In regard to Claim 13, modified method of Tsugita teaches that the ratio of PFPA (5%) to acetic anhydride (20%) would be 1:4 (see page 930, right col. 3<sup>rd</sup> paragraph). In the instant Claim, the lower limit of the ratio is 20:100 or 1:5. Applicant is advised that generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such

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concentration or temperature is critical. "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). Therefore, it would have been obvious to one of ordinary skill in the art to discover the optimum ratio of perfluoroalkanoic acid to alkanoic acid anhydride by routine experimentation.

In regard to Claims 14-16, Tsugita teaches that acetic anhydride is used in the pretreatment step of applying N-acylation protection (see page 930, right col. 3<sup>rd</sup> paragraph).

In regard to Claim 17, Tsugita teaches using acetic anhydride as the alkanoic acid anhydride in the pretreatment step of applying N-acylation protection. Modified method of Tsugita teaches using acetic anhydride in combination with PFPA for the formation of 5-oxazolone and subsequent release of C-terminal amino acids in association with the cleavage of the 5-oxazolone ring (see page 930, right col. 3<sup>rd</sup> paragraph).

### ***Allowable Subject Matter***

7. Claim 7 and claims 8-17 as dependent on claim 7 are objected to as being dependent upon a rejected base claim, but claim 7 would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims, and when claim 7 is allowable, claims 8-17 would be allowable if only depending on claim 7.

The following is a statement of reasons for the indication of allowable subject matter:

Tsugita in view of Covey and Xu, and further in view of Vogt does not teach or fairly suggest any process for C-terminal sequencing in which the reaction for C-terminal stepwise degradation are carried out for the peptide being maintained in a state that it is bound on a polyacrylamide gel.



***Response to Arguments***

8. Applicant's arguments filed 03/16/2010 have been fully considered but they are not persuasive.

Applicant argues that "Tsugita fails to teach or suggest any process for C-terminal sequencing in which the reaction for C-terminal stepwise degradation are carried out for **the peptide being maintained in a state that it is bound on the gel carrier, in particular, on a polyacrylamide gel, as recited in Claims 1, 18** and all claims depending therefrom of the present application." (See Remark, page 33). In response, Examiner can not find the quoted limitation above in Claims 1 and 18. During a phone interview with Mark Cohen on April 14, 2010, Examiner proposes to include the quoted limitation above into Claims 1 and 18, so that Claims 1 and 18 and all claims depending therefrom would have been allowable. No agreement was reached.

Applicant argues that Tsugita fails to provide any evidence suggesting that N-acetylation on the amino group of Lys residue and O-acetylation on the hydroxyl group of Ser and Thr residues as well as N-acetylation on the N-terminus are achieved at the first reaction sub-step. In response, Tsugita teaches pretreatment step using acetic anhydride react with the original dried peptide to acetylate the N-terminus and form an oxazolone at the C-terminal carboxyl group (see page 930, right col. 3<sup>rd</sup> paragraph). While Tsugita does not specifically teach acetylation of the side chain of lysine residue, acetylation of the side chain of lysine residue is the inherent result of reacting acetic anhydride with peptide that has lysine residue.

Applicant argues that Tsugita fails to provide any evidence suggesting that acetic anhydride **with 20% acetic acid tetrahydrofuran solution would be successfully used in the absence of DTT for N-acetylation on the amino group of Lys residue as well as N-acetylation on the N-terminus**. In response, the limitation **tetrahydrofuran** is not in any of the claims.

Applicant argues that "Tsugita fails to teach or suggest any reaction for degradation of the oxazolone-ring which would be achieved without use of methanol (CH<sub>3</sub>OH). Furthermore, Tsugita fails to provide any teaching or suggestion that PEPA (pentafluoropropionic acid: CF<sub>3</sub>CF<sub>2</sub>-COOH) **without methanol (CH<sub>3</sub>OH)** would be used

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as a reactant for the degradation of the oxazolone, in place of PFPMe (pentafluoropropionic methyl ester:  $\text{CF}_3\text{CF}_2\text{-CO-OCH}_3$ ) with methanol ( $\text{CH}_3\text{OH}$ ). Methanol ( $\text{CH}_3\text{OH}$ ) is a well-known protic solvent that is a suitable reactant for alcoholysis reaction. Therefore, Tsugita fails to provide any teaching or suggestion that PEPA (pentafluoropropionic acid:  $\text{CF}_3\text{CF}_2\text{-COOH}$ ) **without any protic solvent such as methanol ( $\text{CH}_3\text{OH}$ )** would be used as a reactant for the degradation of the oxazolone-ring. At the least, Tsugita fails to provide any teaching or suggestion that the degradation of the oxazolone-ring would be made by using the vapor of PFPMe **in the absence of any vapor of protic solvent such as methanol**". In response, the limitation **"without methanol" or "in the absence of any vapor of protic solvent such as methanol"** is not in any of the claims.

Applicant argues that Tsugita by no means uses FAB-MS or MALDI-TOF-MS for the process as disclosed in "2.13 C-terminal sequencing". In response, Tsugita teaches using MALDI-TOF-MS for measuring the molecular weight of the peptide fragments (see page 931, left col. 2<sup>nd</sup> paragraph).

Applicant argues that Tsugita fails to provide any teaching or suggestion that acetic anhydride would be successfully used for degradation of the oxazolone ring in the presence of PFPMe (pentafluoropropionic methyl ester:  $\text{CF}_3\text{CF}_2\text{-CO-OCH}_3$ ). In response, Tsugita teaches allowing acetic anhydride to act on the original dry peptide in the presence of pentafluoropropionic methyl ester (PFPMe) to successively release the C-terminal amino acid by the cleavage of the 5-oxazolone ring (see page 930, right col. 3<sup>rd</sup> paragraph).

The applicant argues that "Covey fails to provide any suggestion as to whether or not such a double charge rule will be also observed for MALDI-TOF-MS or FRB-MS". Although Covey does not specifically teach MALDI-TOF-MS, ordinary skill in the art would know that the double charge rule taught by Covey is true for all ion evaporation mass spectrometry of Tryptic fragments. MALDI-TOF-MS or FRB-MS is not an exception.

Applicant argues that "Xu fails to teach or suggest any use of the protonated molecules  $[\text{M}+\text{H}]^+$  for the positive-ion mode analysis. Accordingly, Xu fails to provide any

teaching or suggestion that the protonated molecules  $[M+H]^+$  will be used for structural characterization of peptides in combination with the deprotonated molecules  $[M-H]^-$ ." In response Xu teaches the positive mode MALDI spectrum in Figure 3 and 4 and negative mode of MALDI spectrum in Figure 5 and 6. Thus, Xu demonstrates that sensitive detection can be achieved for both positive and negative mode for MALDI-MS. Xu teaches that extension of MALDI-TOF-MS allows structural information to be obtained in addition to molecular weight data by means of so called post source decay (PSD) analysis (see page 8, left col. 2<sup>nd</sup> paragraph). Xu shows that fragmentation patterns in positive mode and negative mode PSD were complementary for the elucidation of the peptide chain sequence (see page 10-13, *MALDI-PSD Analysis*).

Applicant argues that Harris fails to provide any experimental evidence suggesting that the  $[M-H]^-$  ions from the trypsin autolysis fragments will be successfully used as mass calibrants in the negative-ion mode MALDI-TOF based analysis. At the least, Harris fails to provide any experimental evidence suggesting that the  $[M-H]^-$  ions from the trypsin autolysis fragments will be successfully measured in the negative-ion mode MALDI-TOF based analysis. In response, the mass (M) of a trypsin autolysis fragment should be the same no matter it is measured in a positive mode  $(M+H)^+$  (M+1) or a negative mode  $(M-H)^-$  (M-1) of MALDI-TOF. The possibility of success of using trypsin autolysis fragments as mass calibrants in the negative-ion mode MALDI-TOF based analysis would have been reasonably expected by one of ordinary skill in the art.

### **Conclusion**

9. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any

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extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROBERT XU whose telephone number is (571)270-5560. The examiner can normally be reached on Mon-Thur 7:30am-5:00pm, Fri 7:30am-4:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Vickie Kim can be reached on (571)272-0579. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

5/6/2010

/Yelena G. Gakh/  
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